

<p align="center">8 OTHER BIOLOGICAL SAMPLES</p>	<p align="center">Page 1 of 9</p>
<p align="center">TRAINING MANUAL: CASE APPROACH AND IDENTIFICATION OF BIOLOGICAL SUBSTANCES</p>	<p>Amendment Designator:</p>
	<p>Effective Date: 14-March-2006</p>
<p>8 OTHER BIOLOGICAL SAMPLES</p> <p>8.1 GOALS</p> <p>8.1.1 To become familiar with the physical and chemical characteristics of vaginal fluid.</p> <p>8.1.2 To learn the theory behind the procedures for the detection of urine and feces, including the specificity and limitations of the tests as well as the use of controls.</p> <p>8.1.3 To become acquainted with the limitations of the Ouchterlony double diffusion test to determine species origin on vaginal, urine, fecal, and tissue samples.</p> <p>8.1.4 To become acquainted with the limitations and specificity of the chemical tests for the presence of amylase in saliva.</p> <p>8.2 TASKS</p> <p>8.2.1 Test several human and animal urine stains of varying dilutions and sizes using the urease test.</p> <p>8.2.2 Test physiological fluid stains (blood, semen, vaginal fluid, saliva, and feces) using the urease test.</p> <p>8.2.3 Test several human and animal fecal stains of varying sizes using Edelman's Test.</p> <p>8.2.4 Test several human vaginal, urine, feces, and tissue samples using Ouchterlony double diffusion. Compare results. Refer to section 6.4.9, Ouchterlony double diffusion, for the procedure.</p> <p>8.2.5 Observe and obtain instruction from qualified examiners performing routine examinations of case material.</p> <p>8.2.6 Read applicable literature. Refer to Appendix A and Appendix B.</p> <p>8.3 TRAINING EVALUATION</p> <p>8.3.1 Knowledge</p> <p>8.3.1.1 Review of notes in training notebook by training coordinator.</p> <p>8.3.1.2 Mini-mock trials/oral and practical examinations.</p> <p>8.3.1.3 Completion of checklist by training coordinator.</p> <p>8.3.2 Skills</p> <p>8.3.2.1 Observation by training coordinator or designee.</p> <p>8.3.2.2 Review of notes in training notebook by training coordinator.</p> <p>8.3.2.3 Mini-mock trials/oral and practical examinations.</p>	

<p align="center">8 OTHER BIOLOGICAL SAMPLES</p>	<p align="center">Page 2 of 9</p>
<p align="center">TRAINING MANUAL: CASE APPROACH AND IDENTIFICATION OF BIOLOGICAL SUBSTANCES</p>	<p>Amendment Designator:</p>
	<p>Effective Date: 14-March-2006</p>
<p>8.3.2.4 Completion of checklist by training coordinator.</p> <p>8.4 DETECTION OF URINE – TECHNICAL NOTES</p> <p>8.4.1 Screening items such as clothing or bedding for the presence of urine stains may be facilitated by the use of an alternate light source (ALS). Alternate light sources include a UV light (sometimes referred to as a “Wood’s Lamp” by Forensic Nurses), the Omnicrome FLS 5000, LumaLite™ 2000A, and Mini Crime Scope MCS400, to name a few. Users must read the directions accompanying each ALS in order to learn the best combination of wavelengths and filters, to avoid damaging the instrument during start up and shut down, and to protect their eyes from the powerful light. The use of appropriate goggles (dependent on the ALS) helps to make the reaction detectable to the eye, while simultaneously protecting the eyes from the light source. If proper eye protection is not worn, permanent damage to the eye may occur. The principle behind the light sources is that urine contains a component (urea) which reacts to light between 450 and 455 nm wavelengths. The reaction appears as a light stain against a dark background. The reaction must be interpreted with caution since other substances (such as, but not limited to, semen, saliva, makeup, yogurt, cleaners, bleach alternatives such as UV dyes) may also react to an ALS. Samples exhibiting a reaction to an ALS require further examination to detect the presence of urine.</p> <p>8.4.2 The urease test is a presumptive test for the presence of urine and is based on the fact that urea is found in high concentration in urine. Although there are many different presumptive tests for the presence of urine, there are no confirmatory tests available for the identification of urine in a dried stain. The urease reagent reacts with urea, releasing ammonia from the stain, which turns red litmus paper to a blue color.</p> <p>8.5 UREASE TEST (Reference 6, pp. 191-193, Appendix B)</p> <p>8.5.1 Equipment</p> <p>8.5.1.1 Scissors</p> <p>8.5.1.2 Tweezers</p> <p>8.5.1.3 Scalpel or other sharp instrument (to cut cork)</p> <p>8.5.1.4 Heat block (37° C)</p> <p>8.5.2 Materials</p> <p>8.5.2.1 Test tubes (10 X 75 mm)</p> <p>8.5.2.2 Corks</p> <p>8.5.2.3 Disposable pipets</p> <p>8.5.3 Reagents</p> <p>8.5.3.1 Distilled water</p> <p>8.5.3.2 Urease reagent</p>	

8 OTHER BIOLOGICAL SAMPLES	Page 3 of 9
TRAINING MANUAL: CASE APPROACH AND IDENTIFICATION OF BIOLOGICAL SUBSTANCES	Amendment Designator:
	Effective Date: 14-March-2006
<div>8.5.3.2.1 Store at 2-8° C.</div> <div>8.5.3.2.2 According to the manufacturer (Sigma), reagents stored at 2-8° C will have a shelf life of 2 years from the manufacturer’s quality control date.</div> <div>8.5.3.3 Red litmus paper</div> <div>8.5.3.4 Positive control (known urine)</div> <div>8.5.4 Minimum Standards and Controls</div> <div>8.5.4.1 A positive reagent control (known urine stain) and a substrate control (or if not available, distilled water) must be tested and results documented in the case file. If the stain is on a cotton swab, it is not necessary to test a substrate control. It is not necessary to test submitted control swabs.</div> <div>8.5.5 Urease Test Procedure</div> <div>8.5.5.1 Cut an approximate 2 cm² piece of a suspected urine stain and the appropriate controls into small pieces. Place the cuttings into appropriately labeled 10 X 75 mm test tubes.</div> <div>8.5.5.2 Add 3-4 drops of distilled water and 6-7 drops of urease reagent to each tube.</div> <div>8.5.5.3 Cut a slit into the small end of a cork and insert a strip of red litmus paper into this slit.</div> <div>8.5.5.4 Place the cork (with red litmus paper) into each test tube. Do not allow the litmus paper to touch the liquid.</div> <div>8.5.5.5 Incubate the samples in a 37° C heat block for 30 minutes.</div> <div>8.5.5.6 Observe any change in the color of the litmus paper. Document results in the case file.</div> <div>8.5.5.7 All controls must give the expected results before a conclusion can be reached on an unknown sample. When all controls work properly and a positive reaction is observed for the unknown sample, urine is <u>indicated</u> to be present.</div> <div>8.5.5.8 Interpretation</div> <div>8.5.5.8.1 Positive Reaction = Red litmus paper turns blue</div> <div>8.5.5.8.2 Negative Reaction = No color change to red litmus paper</div> <div>8.5.5.8.3 Inconclusive Reaction = No color change of the positive control to the red litmus paper and/or substrate control turns red litmus paper blue</div> <div>8.5.5.9 Reporting Results</div> <div>8.5.5.9.1 Report positive test results as “Urine was indicated...”</div>	

8 OTHER BIOLOGICAL SAMPLES	Page 4 of 9
TRAINING MANUAL: CASE APPROACH AND IDENTIFICATION OF BIOLOGICAL SUBSTANCES	Amendment Designator:
	Effective Date: 14-March-2006
<div data-bbox="467 296 1282 331" data-label="Text"> <p>8.5.5.9.2 Report negative test results as “No urine was detected...”</p> </div> <div data-bbox="467 363 1481 399" data-label="Text"> <p>8.5.5.9.3 Report inconclusive test results as “The test for urine was inconclusive...”</p> </div> <div data-bbox="198 430 1019 466" data-label="Section-Header"> <h2>8.6 DETECTION OF FECAL MATERIAL – TECHNICAL NOTES</h2> </div> <div data-bbox="256 497 1557 766" data-label="Text"> <p>8.6.1 Edelman’s test is a presumptive test for the presence of fecal material and is based on the detection of urobilinogen found in high concentration in feces. Urobilinogen is formed in the intestine by the reduction of bilirubin. Urobilinogen is oxidized to urobilin, which is soluble in alcohol. In the presence of neutral alcoholic salts, a green fluorescent complex is formed between urobilin from human or Carnivore feces and zinc. Due to the presence of chlorophyll in Herbivore (ruminants, such as cattle, sheep, and deer) feces, fluorescence will be orange-pink. Although there are other presumptive tests to indicate the presence of fecal material, there are no confirmatory tests available for the identification of fecal material.</p> </div> <div data-bbox="198 798 940 833" data-label="Section-Header"> <h2>8.7 EDELMAN’S TEST (Reference 12, pp. 4-7, Appendix B)</h2> </div> <div data-bbox="256 865 602 900" data-label="Section-Header"> <h3>8.7.1 Safety Considerations</h3> </div> <div data-bbox="350 932 1557 1167" data-label="List-Group"> <ul style="list-style-type: none"> 8.7.1.1 Mercuric chloride - Caution! Very toxic if inhaled or swallowed, or if in contact with skin! Poisonous! Dangerous! May be fatal! 8.7.1.2 Zinc chloride - Caution! Corrosive! 8.7.1.3 Amyl alcohol (isopentyl alcohol) - Caution! Harmful if swallowed or inhaled! Irritant! Combustible! </div> <div data-bbox="256 1199 474 1234" data-label="Section-Header"> <h3>8.7.2 Equipment</h3> </div> <div data-bbox="350 1266 945 1568" data-label="List-Group"> <ul style="list-style-type: none"> 8.7.2.1 Scissors 8.7.2.2 Tweezers 8.7.2.3 Centrifuge 8.7.2.4 Long wavelength ultraviolet light source 8.7.2.5 Vortex </div> <div data-bbox="256 1602 454 1635" data-label="Section-Header"> <h3>8.7.3 Materials</h3> </div> <div data-bbox="350 1669 933 1772" data-label="List-Group"> <ul style="list-style-type: none"> 8.7.3.1 Disposable pipets 8.7.3.2 Test tubes and/or microcentrifuge tubes </div> <div data-bbox="256 1801 451 1837" data-label="Section-Header"> <h3>8.7.4 Reagents</h3> </div> <div data-bbox="350 1869 1310 1904" data-label="List-Group"> <ul style="list-style-type: none"> 8.7.4.1 10% Saturated mercuric chloride solution (1 g in 10 ml of 95% ethanol) </div>	

8 OTHER BIOLOGICAL SAMPLES	Page 5 of 9
TRAINING MANUAL: CASE APPROACH AND IDENTIFICATION OF BIOLOGICAL SUBSTANCES	Amendment Designator:
	Effective Date: 14-March-2006
<div>8.7.4.2 10% Saturated zinc chloride solution (1 g in 10 ml of 95% ethanol)</div> <div>8.7.4.3 Amyl alcohol (isopentyl alcohol)</div> <div>8.7.4.4 Distilled water</div> <div>8.7.4.5 Positive control (known feces)</div> <div>8.7.5 Storage</div> <div>8.7.5.1 The 10% saturated solutions of mercuric chloride and zinc chloride are stable at room temperature.</div> <div>8.7.6 Labeling</div> <div>8.7.6.1 Label each bottle with the contents and lot number (date of preparation followed by the initials of person preparing the solution). Example: 10% saturated zinc chloride solution Lot Number 100899JD was prepared by Jane Doe on October 8, 1999.</div> <div>8.7.6.2 There is no expiration date (see 8.7.7 Minimum Standards and Controls).</div> <div>8.7.7 Minimum Standards and Controls</div> <div>8.7.7.1 A positive reagent control (known fecal stain), and a substrate control (when available) must be tested and results documented in the case file. Distilled water will be used as a negative control. If the stain is on a cotton swab, it is not necessary to test a substrate control. It is not necessary to test submitted control swabs.</div> <div>8.7.8 Edelman’s Test Procedure</div> <div>8.7.8.1 Place an approximate ½ cm² piece of suspected fecal stain and controls in appropriately labeled test tubes or microcentrifuge tubes and extract in a minimum of 3 drops of distilled water at room temperature for at least 15 minutes.</div> <div>8.7.8.2 Remove the material and add a minimum of 3 drops of 10% saturated zinc chloride solution to the extract.</div> <div>8.7.8.3 Add 5 drops of amyl alcohol (isopentyl alcohol) to the extract and vortex.</div> <div>8.7.8.4 Centrifuge for 5 minutes. Pipet the supernatant layer into an appropriately labeled test tube.</div> <div>8.7.8.5 Add 3 drops of 10% saturated mercuric chloride solution.</div> <div>8.7.8.6 Observe color changes in both white and ultraviolet light. Document results. If urobilin is present the solution may become rose-pink, but will show a crab apple green fluorescence under long wave ultraviolet light.</div>	

<p align="center">8 OTHER BIOLOGICAL SAMPLES</p>	<p align="center">Page 6 of 9</p>
<p align="center">TRAINING MANUAL: CASE APPROACH AND IDENTIFICATION OF BIOLOGICAL SUBSTANCES</p>	<p align="center">Amendment Designator:</p>
	<p align="center">Effective Date: 14-March-2006</p>
<p>8.7.8.7 All controls must give the expected results before a conclusion can be reached on an unknown sample. When all controls work properly and a positive reaction is observed for the unknown sample, feces is <u>indicated</u> to be present.</p> <p>8.7.8.8 Interpretation</p> <p>8.7.8.8.1 Positive Reaction = Crab apple green fluorescence under long wave ultraviolet light</p> <p>8.7.8.8.2 Negative Reaction = No green fluorescence under long wave ultraviolet light</p> <p>8.7.8.8.3 Inconclusive Reaction = No green fluorescence of the positive control under long wave ultraviolet light and/or substrate control exhibits crab apple green fluorescence under long wave ultraviolet light</p> <p>8.7.8.9 Reporting Results</p> <p>8.7.8.9.1 Report positive results as “Fecal material was indicated...”</p> <p>8.7.8.9.2 Report negative results as “No fecal material was detected...”</p> <p>8.7.8.9.3 Report inconclusive results as “The test for fecal material was inconclusive...”</p>	
<p>8.8 DETECTION OF SALIVA – TECHNICAL NOTES</p>	
<p>8.8.1 Screening items such as masks or clothing for the presence of saliva stains may be facilitated by the use of an alternate light source (ALS). Alternate light sources include a UV light (sometimes referred to as a “Wood’s Lamp” by Forensic Nurses), the Omnichrome FLS 5000, LumaLite™ 2000A, and Mini Crime Scope MCS400, to name a few. Users must read the directions accompanying each ALS in order to learn the best combination of wavelengths and filters, to avoid damaging the instrument during start up and shut down, and to protect their eyes from the powerful light. The use of appropriate goggles (dependent on the ALS) helps to make the reaction detectable to the eye, while simultaneously protecting the eyes from the light source. If proper eye protection is not worn, permanent damage to the eye may occur. The principle behind the light sources is that biological fluids may react to light between 450 and 455 nm wavelengths. The reaction may either appear as a faint light stain against a dark background, or in some circumstances, the stain appears darker against a light background. The reaction must be interpreted with caution since other substances (such as, but not limited to, urine, semen, makeup, yogurt, cleaners, bleach alternatives such as UV dyes) may also react to an ALS. Since the Department does not conduct presumptive testing for the presence of saliva, samples exhibiting a reaction to an ALS may require DNA analysis.</p>	

8 OTHER BIOLOGICAL SAMPLES	Page 7 of 9
TRAINING MANUAL: CASE APPROACH AND IDENTIFICATION OF BIOLOGICAL SUBSTANCES	Amendment Designator:
	Effective Date: 14-March-2006
<p style="text-align: center;">OTHER BIOLOGICALS STUDY QUESTIONS</p> <ol style="list-style-type: none"> 1 Describe the mechanism for the Urease test, including chemicals used and why. 2 Describe the mechanism for Edelman's test including chemicals used and why. 3 Are there any false positives for the Urease test? Edelman's test? 4 When would you test evidence for urine? Feces? 5 In what cases does the finding of urine become important? Feces? 6 What are some other methods used for the detection of urine and feces? 7 Name at least one method that can be used to indicate the presence of saliva. Why doesn't DFS test for saliva? 8 Is there a test to indicate the presence of vaginal fluid? If there is such a test, why doesn't DFS use the test? 9 You receive a piece of bone and a piece of tissue from a decomposed body. How do you preserve these samples for possible future testing? 10 You get a call from an investigator saying he has what appears to be a piece of scalp tissue on broken glass at a felonious assault scene. What do you tell him to do with regard to packaging it and submitting it to the lab? 	

8 OTHER BIOLOGICAL SAMPLES	Page 8 of 9
TRAINING MANUAL: CASE APPROACH AND IDENTIFICATION OF BIOLOGICAL SUBSTANCES	Amendment Designator:
	Effective Date: 14-March-2006

CHECKLIST FOR OTHER BIOLOGICAL SAMPLES

Name of Trainee: _____

1. Tested several human and animal urine stains of varying dilutions and sizes using the Urease Test.

Date: _____ Training Coordinator: _____

Comments: _____
2. Tested physiological fluid stains (blood, semen, vaginal fluid, saliva, and feces) using the Urease Test.

Date: _____ Training Coordinator: _____

Comments: _____
3. Tested several human and animal fecal stains of varying sizes using Edelman's Test.

Date: _____ Training Coordinator: _____

Comments: _____
4. Tested several human vaginal, urine, feces and tissue samples using Ouchterlony double diffusion.

Date: _____ Training Coordinator: _____

Comments: _____
5. Trainee has learned the theory behind the techniques used for the detection of urine and feces, including specificity and limitations of the tests as well as the use of controls.

Date: _____ Training Coordinator: _____

Comments: _____
6. Trainee has learned the limitations of the Ouchterlony double diffusion test to determine species origin on vaginal fluid, urine, fecal, and tissue samples.

Date: _____ Training Coordinator: _____

Comments: _____
7. Trainee's notebook is organized and complete.

Date: _____ Training Coordinator: _____

Comments: _____

8 OTHER BIOLOGICAL SAMPLES	Page 9 of 9
TRAINING MANUAL: CASE APPROACH AND IDENTIFICATION OF BIOLOGICAL SUBSTANCES	Amendment Designator:
	Effective Date: 14-March-2006
<p>8. Trainee has participated in a mock trial and/or a practical or oral examination. Performance was satisfactory.</p> <p>Date:_____ Training Coordinator:_____</p> <p>Comments:_____</p> <p>9. Trainee has read and understands all applicable literature.</p> <p>Date:_____ Training Coordinator:_____</p> <p>Comments:_____</p> <p style="text-align: right;">◆END</p>	